COMMENTARY
PRKA/AMPK: Integrating Energy Status with Fertility in Pituitary Gonadotrophs
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Polycystic ovarian syndrome (PCOS) is a common reproductive disorder, characterized by irregular menses, hyperandrogenism, and polycystic ovaries. Patients with PCOS also commonly suffer from infertility, obesity, and insulin resistance [1]. The biochemical changes that are commonly associated with PCOS are elevated LH to FSH ratios, elevated bioavailable and total testosterone, and elevated insulin levels associated with insulin resistance. Historically considered a hyperandrogenic disease, the treatments for PCOS focused on weight reduction and alleviating the symptoms associated with excess androgens when pregnancy was not desired or the induction of ovulation with the antiestrogen, clomiphene citrate, when pregnancy was desired. However, effective treatment of PCOS symptoms with insulin sensitizing agents for the treatment of Type II diabetes has resulted in an increasing emphasis being placed on insulin resistance in the pathogenesis of this disease [2].

Currently, the biguanide insulin sensitizing agent, metformin, is increasingly used to both alleviate the symptoms of PCOS and to treat the associated infertility. The successful use of this therapy has resulted in a closer examination of the mechanism of action of metformin at targets throughout the hypothalamic-pituitary-gonadal axis. In the treatment of Type II Diabetes, metformin decreases hepatic glucose production and increases glucose consumption in peripheral tissues resulting in a decrease in blood glucose levels, decreased insulin release, and decreased fat accumulation [3]. At the cellular level, metformin inhibits the mitochondrial electron transport chain at complex I to decrease ATP production; the resulting increase in AMP levels activates the AMP dependent kinase (AMPK, official symbol PRKA). PRKA serves as a regulator of cellular and systemic energy homeostasis [3]. AMP kinase is a heterotrimeric protein consisting of alpha, beta, and gamma subunits. It can be activated by any cellular stress that increases AMP levels by means of the allosteric binding of AMP to sites in the gamma subunit [4], as well as through phosphorylation of Thr172 in the alpha subunit by serine/threonine kinase 11(STK11/LKB1), calcium/calmodulin dependent protein kinase kinase (CAMKK), and most recently the TGFβ-activated kinase (TAK1) [4-6]. A number of hormones, (leptin, ghrelin, adiponectin, resistin) that regulate energy homeostasis have also been shown to exhibit tissue specific stimulation or inhibition of AMP kinase [4, 7]. Treatment with metformin may improve reproductive health through a combination of effects on multiple organ systems, resulting in increased insulin sensitivity and the normalization of insulin, IGF1 and androgen levels.

The study by Tosca et al. [8] provides a novel point of integration between mediators of energy homeostasis and the regulation of reproductive function at the level of the pituitary gland. Using both pharmacologic and molecular tools, the authors have presented compelling data to link this energy sensing pathway to both activin and GNRH mediated regulation of gonadotropin expression [8].

Since PCOS is associated with elevated LH levels, the authors first asked if metformin treatment could alter the secretion of LH and FSH. Using primary rat pituitary cultures, these investigators determined that metformin had no effect on its own, but was able to inhibit LH release in response to GNRH. FSH release was inhibited by metformin in response to both GNRH and activin. Because metformin activates AMP kinase, the authors examined the expression of the various AMP kinase subunits in rat pituitaries and primary cultures of rat pituitary cells. They assessed expression of the 6 PRKA subunits by RT-PCR and western blot analysis. They used immunofluorescent analysis to assess the expression of the alpha 1 subunit of PRKA and co-localize its expression with protein hormone expression from each of the five cell types in the anterior pituitary gland. This subunit was strongly expressed in gonadotropes and thyrotropes, less in somatotropes and lactotropes, and was undetectable in

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corticotropes. An earlier study indicated that corticotropes primarily express the alpha 2 subunit of the kinase. As a negative control, they showed that there was no staining in *Prkaa1* knockout mice pituitaries.

The authors next examined the effect of metformin on PRKAA activating phosphorylation and a downstream target of PRKA, acetyl-CoA carboxylase. They demonstrate dose and time dependent changes in phosphorylation of these targets. Using compound C, a specific inhibitor of PRKA activity, they verified the role of PRKA in the inhibition of LH and FSH release by metformin. Similarly, a dominant negative PRKA construct also blocked the ability of metformin to inhibit *Fshb* mRNA transcription in response to activin A.

The role of SMAD2, AKT, and the MAPK14 (p38) and MAPK3/1 (Erk1/2) MAPkinases in activin A and GNRH signaling was examined and a time course of response for those activators was established. Having validated this model, they determined that metformin treatment is able to block activation of these signaling pathways, and this effect is reversed by the dominant negative PRKA construct. Thus, PRKA activation by metformin inhibits the activation of these critical gonadotrope signaling pathways. Since activin signaling is an important regulator of GNRH receptor, as well as *FSHB* gene expression [9], the activation of PRKA by metformin and subsequent inhibition of activin signaling can also reduce the sensitivity of gonadotropes to GNRH stimulation.

While metformin acts at multiple sites that may directly or indirectly alter gonadotropin regulation and fertility, metformin induction of adenosine 5’ monophosphate-activated protein kinase (AMPK/PRKA) is a potential point for regulation of fertility at the level of the pituitary. The PRKA complex is increasingly recognized as a critical regulator of cellular and systemic energy homeostasis [4]. The fact that PRKA can be activated allosterically by increased AMP/ATP ratios, as well as through phosphorylation of the α-subunit in response to neuroendocrine stimuli, means that PRKA can elicit cellular responses to local energy levels as well as integrate hormonal signals of systemic energy status at distal targets. For example, the differential regulation of PRKA activity by leptin and ghrelin contributes to appetite control in the hypothalamus and glucose regulation in the liver. In addition, the insulin sensitizing hormone, adiponectin, acting in a manner similar to metformin, stimulates PRKA activity to block fat accumulation in adipocytes, gluconeogenesis in the liver and stimulates glucose uptake in the muscles [4].

At the level of the pituitary gland, adiponectin, through activation of PRKA decreases LH synthesis and secretion in the mouse gonadotrope derived LβT2 cell line [10] (Figure 1). In addition to acting at the level of the hypothalamus to stimulate the reproductive axis, leptin also increases gonadotropin synthesis and secretion in response to GNRH stimulation [11-13]. The established regulation of PRKA in response to leptin signaling suggests that PRKA may also play a role in these events (Figure 1). While additional studies are clearly required to understand the interplay of activating and inhibitory signals with the multiple subunits of the PRKA complex, the current study establishes the expression of PRKA subunits in primary pituitary gonadotropes and provides a mechanism through which metformin activation of PRKA can directly impact the signaling pathways involved in gonadotropin synthesis and secretion (Figure 1). Finally, the differential regulation of adenosine 5’ monophosphate-activated protein kinase by adipokines provides a point of integration of cellular and systemic energy with reproduction at the level of the pituitary.
REFERENCES


FIGURE LEGEND

Figure 1. Adenosine 5’ monophosphate-activated protein kinase (PRKA/AMPK) is a potential point of integration of cellular and systemic energy with reproduction at the level of the pituitary. The activation of AMP kinase by cellular stress through elevated AMP levels or by metformin and adiponectin by phosphorylation at Thr172 by an AMPK kinase (STK11/LKB1, CAMKK, TAK1) blocks Activin and GNRH signaling in gonadotropes to alter gonadotrope sensitivity and LH and FSH synthesis and secretion. Leptin may stimulate gonadotropin synthesis and secretion by inhibiting the activation of PRKA. Arrows indicate stimulated pathways; lines terminating in a black ball indicate inhibition.